

SUPPLEMENTARY INFORMATION FOR:

mRNA Vaccination Induces Durable Immune Memory to SARS-CoV-2 with Continued Evolution to Variants of Concern

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Figure S1. Gating strategy for SARS-CoV-2-specific memory B cells. For **panel 1**, lymphocytes were first identified based on forward- and side-scatter from bulk PBMC samples. Singlets were excluded by FSC-A/FSC-H and FSC-A/FSC-W. Dead cells were excluded using Ghost 510 viability dye. Total B cells were then identified as CD3- CD19+ cells. Naïve B cells were identified as IgD+ CD27- B cells and excluded from downstream analysis. Memory B cells were subsequently identified from non-naïve B cells as CD20+ CD38lo/int cells. A BV711 decoy probe was used to gate out memory B cells that non-specifically bound streptavidin. Spike- and HA-binding were then quantified on decoy- memory B cells. Binding to RBD probe was also measured on Spike+ memory B cells. IgG, IgM, and IgA isotypes were evaluated for both Spike+ and Spike+ RBD+ memory B cells. CD71 was measured as an activation marker on Spike+ memory B cells. For **panel 2**, total B cells were enriched by negative selection from PBMC samples prior to staining. Live, non-naïve B cells were identified as described above. Plasmablasts were then identified as CD27+ CD38+ non-naïve B cells and were excluded from downstream analysis. Decoy- cells were excluded as described above. Spike- and nucleocapsid-specific B cells were identified based on binding to corresponding probes. Spike+ memory cells were then analyzed for co-binding to N-terminal domain (NTD) or S2 domain probes. Memory B cells that were Spike+ but NTD- and S2- were subsequently analyzed for co-binding to a panel of variant RBD probes, including wild-type (WT), B.1.1.7, B.1.351, and B.1.617.2 RBDs. IgG expression was evaluated for all antigen-specific populations.

Figure S2. Class-switching of Spike+ and Spike+ RBD+ memory B cells after mRNA vaccination. **A)** Percent IgG+ of Spike+ and **B)** Spike+ RBD+ memory B cells over time after mRNA vaccination. **C)** Summary statistics for % IgG+, % IgM+, and % other isotype+ of SARS-CoV-2-specific memory B cells over time. Outer rings represent total Spike+ memory B cells, inner rings represent Spike+ RBD+ memory B cells.

Figure S3. Extended analysis of SARS-CoV-2-specific memory B cell frequencies and class-switching after mRNA vaccination. **A)** Frequency of SARS-CoV-2 antigen-specific memory B cells over time after mRNA vaccination or infection. Data are represented as a percentage of total B cells. **B)** Cross sectional analysis of antigen-specific memory B cell frequencies at 6 months post-vaccination or sero-positivity. Pre-immune baseline samples are also shown as a control. **C)** Class-switching of RBD-binding cells over time after mRNA vaccination or infection. Data are represented as the percent of cells that are IgG+. **D)** Cross sectional analysis of class-switching to IgG at 6 months post-vaccination or sero-positivity.

Figure S4. Extended analysis of variant-binding BCR sequencing data. **A)** Individual flow plots of sorted memory B cell populations in 8 SARS-CoV-2 naïve and 4 SARS-CoV-2 recovered individuals. **B)** Histogram of VH gene usage across different antigen binding populations. Data are represented as a percentage of the overall clones for a given antigen-binding population. **C)** Individual SHM distributions of memory B cell clones for SARS-CoV-2 naïve and recovered individuals. Data are represented as the percent of mutated VH gene nucleotides. **D)** Venn diagram of clonal lineages that are shared between RBD-, WT RBD+ and RBD++ populations. Data were filtered based on larger clones with $\geq 50\%$ mean copy number frequency in each sequencing library.

Figure S5. Gating strategy for SARS-CoV-2-specific memory T cells. Lymphocytes were first identified based on forward- and side-scatter from bulk PBMC samples. Singlets were excluded by FSC-A/FSC-H and SSC-A/SSC-H. Total T cells were identified as Live/Dead- CD3+. CD4 and CD8 T cells were then identified from total T cells. For both CD4+ and CD8+ T cells, naïve cells were identified as CD45RA+ CD27+ and excluded from downstream analysis. Memory subsets were defined based on a combination of CD45A, CD27, and CCR7 expression. CD4+ helper subsets were defined based on CCR6, CXCR3, and CXCR5 chemokine receptor expression. AIM+ CD4+ T cells were identified based on co-expression of CD40L and CD200. AIM+ CD8+ T cells were identified based on co-expression of at least 4 of 5 activation induced markers (intracellular IFN- γ , 41BB, CD40L, CD107a, CD200).

Figure S6. Immune correlations after mRNA vaccination. **A)** Relationship between age and overall vaccine response in SARS-CoV-2 naïve subjects. 6 month post-vaccination samples are colored by age and projected onto the UMAP coordinates from figure 6. **B)** Correlation between individual immune parameters and age at 6 months post-vaccination in SARS-CoV-2 naïve

subjects. **C)** Relationship between sex and overall vaccine response in SARS-CoV-2 naïve subjects. 6 month post-vaccination samples are colored by sex and projected onto the UMAP coordinates from figure 6. **D)** Correlation between T cell responses and variant-specific humoral responses over time in SARS-CoV-2 naïve subjects. **E)** Correlation between peak antibody, memory B, and memory T cell responses 1 week after the second vaccine dose with later responses at 3 and 6 months post-vaccination in SARS-CoV-2 naïve subjects. All statistics were calculated using non-parametric Spearman rank correlation.

		SARS-CoV-2 Naïve	SARS-CoV-2 Recovered	Natural Infection (HCW)
Total	Number	45	16	19
	Average (Years)	36.9	38.3	35.2
	20-30	15	4	8
Age	30-40	14	6	7
	40-50	9	2	2
	50+	7	4	2
Sex	Male	21	10	5
	Female	24	6	14
Race/Ethnicity	White - Non-Hispanic/Latino	27	7	17
	White - Hispanic/Latino	4	1	0
	Asian	8	6	1
	Black	2	1	1
	Native	0	1	0
	Other	1	0	0
Vaccine Type	Pfizer	42	12	--
	Moderna	3	4	--

Table S1. Demographic Information for University of Pennsylvania Healthy COVID Vaccine and Healthcare Worker (HCW) Sero-Monitoring Studies. Number of participants, age, sex, race/ethnicity, and vaccine type are indicated.

Sample ID	Recovered	Population	Total DNA (ng)	Input DNA (ng)	Copies	Unique	In Frame	# Clones	Average VH Identity	CDR3 Length (NT)
N1	No	Naïve B	4160	200	140801	113799	0.841876	14242	0.984176	55.05961
N1	No	Spike+ RBD-	38.64	35.88	164565	131819	0.852932	6684	0.959396	52.42774
N1	No	Spike+ WT RBD+	10.248	9.516	237325	121844	0.829343	2297	0.96788	50.98389
N1	No	Spike+ RBD++	3.892	3.614	212006	106777	0.808652	1803	0.957687	50.4376
N2	No	Naïve B	3848	200	160856	136888	0.82964	18772	0.981181	56.12348
N2	No	Spike+ RBD-	50.4	46.8	138341	115801	0.86341	7043	0.949157	55.41389
N2	No	Spike+ WT RBD+	22.82	21.19	185765	121679	0.827671	4474	0.969472	55.88869
N2	No	Spike+ RBD++	6.188	5.746	193081	100240	0.83583	1669	0.954832	53.36968
N3	No	Naïve B	4108	200	166692	133169	0.836719	15507	0.982458	55.35513
N3	No	Spike+ RBD-	22.26	20.67	159970	104626	0.855542	5891	0.956951	52.64828
N3	No	Spike+ WT RBD+	32.2	29.9	172876	127480	0.849711	6401	0.966868	54.50133
N3	No	Spike+ RBD++	3.976	3.692	176383	88649	0.823144	1374	0.961225	51.42868
N4	No	Naïve B	3406	200	201283	153755	0.822365	15363	0.986036	57.14008
N4	No	Spike+ RBD-	50.4	46.8	205448	149192	0.823989	5119	0.969826	54.63098
N4	No	Spike+ WT RBD+			195133	34123	0.700326	307	0.970296	54.71987
N4	No	Spike+ RBD++	4.256	3.952	235928	95887	0.796986	1128	0.960086	51.63564
N5	No	Naïve B	532	200	151633	117639	0.853735	20456	0.962536	53.97961
N5	No	Spike+ RBD-			11048	2265	0.571429	21	0.954252	59.66667
N5	No	Spike+ WT RBD+	5.768	5.356	130442	52200	0.812448	1205	0.965277	54.18755
N5	No	Spike+ RBD++			148307	21122	0.681373	204	0.974878	51.85294
N6	No	Naïve B	535.5	200	206026	166095	0.848853	19180	0.97315	54.13264
N6	No	Spike+ RBD-	5.292	4.914	180711	85411	0.819632	1630	0.975975	53.02393
N6	No	Spike+ WT RBD+	4.984	4.628	243434	85176	0.814755	1247	0.97978	51.36889
N6	No	Spike+ RBD++			145249	37847	0.759709	412	0.979334	52.58981
N7	No	Naïve B	483	200	222335	177357	0.852768	22040	0.971328	53.32114
N7	No	Spike+ RBD-	2.576	2.392	277624	98253	0.789958	1195	0.976346	50.7046
N7	No	Spike+ WT RBD+	8.092	7.514	286754	140707	0.801635	2324	0.974052	52.3352
N7	No	Spike+ RBD++	1.764	1.638	303988	71141	0.773611	720	0.979057	49.67778
N8	No	Naïve B	343	127.4	133517	103687	0.861062	13632	0.972975	53.99831
N8	No	Spike+ RBD-			195913	44220	0.665782	377	0.97288	55.687
N8	No	Spike+ WT RBD+			131695	28512	0.8	275	0.969267	50.82182
N8	No	Spike+ RBD++			19648	3645	0.815789	76	0.970741	45.21053
R1	Yes	Naïve B	5564	200	166874	129000	0.837699	24054	0.985936	55.67278
R1	Yes	Spike+ RBD-	24.5	22.75	184946	100096	0.851991	3466	0.945325	53.27986
R1	Yes	Spike+ WT RBD+	25.76	23.92	177969	107237	0.826538	5494	0.979587	56.57117
R1	Yes	Spike+ RBD++			191869	38316	0.699115	339	0.982212	54.56637
R2	Yes	Naïve B	3666	200	150269	135921	0.861352	46535	0.985534	53.59173
R2	Yes	Spike+ RBD-	21.14	19.63	156519	99743	0.864045	4222	0.955645	53.29725
R2	Yes	Spike+ WT RBD+	3.192	2.964	249006	86313	0.811163	1075	0.963926	54.00837
R2	Yes	Spike+ RBD++			254772	64204	0.843662	710	0.95905	53.49437
R3	Yes	Naïve B	5239	200	243860	191104	0.841811	31671	0.986992	56.29873
R3	Yes	Spike+ RBD-	24.22	22.49	133725	83873	0.863046	3848	0.950538	51.55249
R3	Yes	Spike+ WT RBD+	7.588	7.046	272563	120491	0.818267	2124	0.970827	55.60734
R3	Yes	Spike+ RBD++			245955	60802	0.709677	589	0.987109	53.60272
R4	Yes	Naïve B	4186	200	193799	155701	0.83858	22680	0.981337	55.67928
R4	Yes	Spike+ RBD-	24.78	23.01	195890	119951	0.849797	3948	0.955369	53.78343
R4	Yes	Spike+ WT RBD+	14.7	13.65	209686	105495	0.846287	3474	0.967402	54.31031
R4	Yes	Spike+ RBD++	5.936	5.512	160571	59064	0.851287	1049	0.95557	52.45281

Table S2. BCR Sequencing Metadata. B cell receptor sequencing metadata. Sample ID indicates subject; Recovered indicates prior COVID-19. Two independent PCR amplifications (biological replicates) were performed for each sample; Input DNA per replicate; Number of valid sequence copies (passing length and other QC filters, see methods); Clones are defined as sequences that share the same VH, JH, CDR3 length and are at least 85% identical in the third complementarity determining region (CDR3) amino acid sequence; Clones with only 1 copy at the subject level are excluded; Average VH identity compared to the nearest germline VH gene (average identity was calculated for each clone and then averaged across clones with each clone counted once per sample); CDR3 length in nucleotides (nt). Productive rearrangements only.

Reagent	Vendor	Identifier	Concentration
Panel 1 - B Cell Probe			
SARS-CoV-2 Biotinylated Full Length Spike	R&D Systems	BT10549-050	200ng
SARS-CoV-2 Biotinylated Full Length Spike	R&D Systems	BT10500-050	25ng
HA(ΔTM)(A/Brisbane/02/2018)(H1N1)	Immune Tech	IT-003-00110ΔTMp	50ng
HA(ΔTM)(B/Colorado/06/2017)	Immune Tech	IT-003-B21ΔTMp	50ng
BV421 Streptavidin	Biolegend	405226	20ng
BV711 Streptavidin	BD Biosciences	563262	20ng
PE Streptavidin	Biolegend	405203	16ng
APC Streptavidin	Biolegend	405207	12.5ng
Ghost Viability Dye Violet 510	Tonbo	13-0870-T100	1:600
BUV563 anti-CD3	BD Biosciences	748569	1:200
BV750 anti-CD19	Biolegend	302262	1:100
BUV805 anti-CD20	BD Biosciences	612905	1:500
BUV395 anti-CD27	BD Biosciences	563815	1:200
BUV661 anti-CD38	BD Biosciences	612969	1:200
APC-H7 anti-CD71	BD Biosciences	563671	1:50
FITC anti-IgA	Miltenyi	130-113-475	1:400
BV480 anti-IgD	BD Biosciences	566138	1:50
PE-Cy7 anti-IgG	Biolegend	410722	1:400
PerCP/Cy5.5 anti-IgM	Biolegend	314512	1:400
Panel 2 - Variant B Cell Probe			
SARS-CoV-2 Biotinylated Full Length Spike	R&D Systems	AVI10549-050	200ng
SARS-CoV-2 Biotinylated RBD	Acro Biosystems	SPD-C82E9-25ug	25ng
SARS-CoV-2 Biotinylated RBD (N501Y)	Acro Biosystems	SPD-C82E6-25ug	25ng
SARS-CoV-2 Biotinylated RBD (K417N/E484K/N501Y)	Acro Biosystems	SPD-C82E5-25ug	25ng
SARS-CoV-2 Biotinylated RBD (L452R/K478N)	Acro Biosystems	SPD-C82Ed-25ug	25ng
SARS-CoV-2 Biotinylated N-Terminal Domain	Sino Biological	40591-V49H-B	30ng
SARS-CoV-2 Biotinylated S2	Acro Biosystems	S2N-C52E8-25ug	50ng
SARS-CoV-2 Biotinylated Nucleocapsid	R&D Systems	BT10474-050	50ng
BV421 Streptavidin	Biolegend	405226	20ng
BV605 Streptavidin	Biolegend	405229	14ng
BV711 Streptavidin	BD Biosciences	563262	12.5ng
BV786 Streptavidin	BD Biosciences	563858	12ng
BUV615 Streptavidin	BD Biosciences	613013	12.5ng
BUV737 Streptavidin	BD Biosciences	612775	12ng
BB515 Streptavidin	BD Biosciences	564453	12.5ng
PE Streptavidin	Biolegend	405203	12.5ng
APC Streptavidin	Biolegend	405207	12.5ng
Ghost Viability Dye Violet 510	Tonbo	13-0870-T100	1:600
BUV563 anti-CD3	BD Biosciences	748569	1:200
BV750 anti-CD19	Biolegend	302262	1:100
BUV395 anti-CD27	BD Biosciences	563815	1:200
BUV661 anti-CD38	BD Biosciences	612969	1:200
BV480 anti-IgD	BD Biosciences	566138	1:50
APC-H7 anti-IgG	BD Biosciences	561297	1:100
Panel 3 - Variant-Specific B Cell Sorting			
SARS-CoV-2 Biotinylated Full Length Spike	R&D Systems	BT10549-050	200ng
SARS-CoV-2 Biotinylated RBD	Sino Biological	40592-V08B-B	25ng

SARS-CoV-2 Biotinylated RBD (K417N/E484K/N501Y)	Sino Biological	40592-V08H85-B	25ng
BV421 Streptavidin	Biolegend	405226	20ng
AF488 Streptavidin	Biolegend	405235	20ng
PE Streptavidin	Biolegend	405203	12.5ng
APC Streptavidin	Biolegend	405207	12.5ng
Ghost Viability Dye Violet 510	Tonbo	13-0870-T100	1:600
APC-Cy7 anti-CD19	BD Biosciences	557791	1:200
BV650 anti-CD20	Biolegend	302336	1:200
BV785 anti-CD27	Biolegend	302832	1:66
PE-Cy7 anti-CD38	eBioscience	25-0389-42	1:200
PE-CF594 anti-IgD	BD Biosciences	562540	1:50

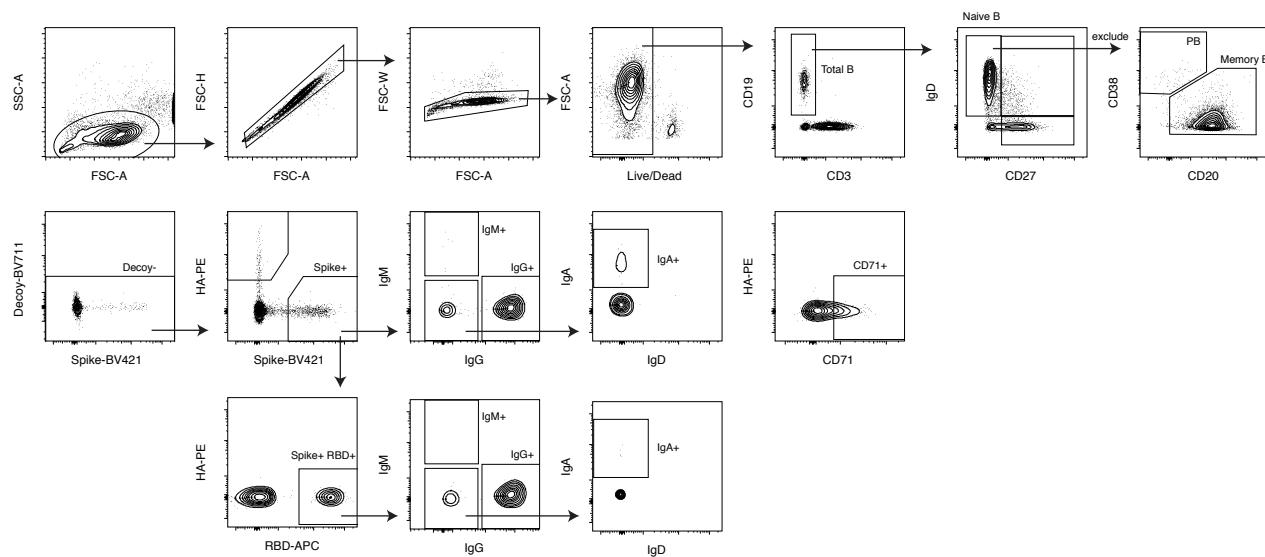
Table S3. Reagents for Memory B Cell Analysis. Reagent, vendor, catalog number, and concentration/dilution are indicated.

Reagent	Vendor	Identifier
Flow Cytometry Antibodies		
BUV395 CD4	BD Biosciences	Cat#563550
BUV496 CD8	BD Biosciences	Cat#612943
BUV615 CD45RA	BD Biosciences	Cat#751555
BUV737 CD27	BD Biosciences	Cat#612829
BUV805 CD3	BD Biosciences	Cat#612896
BV421 CXCR3	Biolegend	Cat#353716
BV650 CCR7	Biolegend	Cat#353234
BV605 CD69	Biolegend	Cat#310938
BV711 CD40L	Biolegend	Cat#310838
BV785 CD107a	Biolegend	Cat#328644
FITC IFNy	Biolegend	Cat#502515
PE CD200	Biolegend	Cat#399804
PE-Cy7 OX40	Biolegend	Cat#350012
AF647 41BB	Biolegend	Cat#309810
APC-R700 CXCR5	BD Biosciences	Cat#565191
APC-Cy7 CCR6	Biolegend	Cat#353432
Peptides		
CD4-S peptide Megapool	Synthetic Biomolecules (aka A&A)	http://www.syntheticbiomolecules.com/
CD8-E peptide Megapool	Synthetic Biomolecules (aka A&A)	http://www.syntheticbiomolecules.com/
Other		
Ghost Dye Violet 510	Tonbo	Cat#13-0870-T500
GolgiStop (Containing Monensin)	BD Biosciences	Cat#51-2092K7
CD40 Antibody, anti-human, pure-functional grade	Miltenyi Biotech	Cat#130-094-133
Anti-Human CD28/CD49d Purified	BD Biosciences	Cat#347690
Human TruStain FcX™ (Fc Receptor Blocking Solution)	Biolegend	Cat#422302
Foxp3 / Transcription Factor Fixation/Permeabilization Concentrate and Diluent	eBioscience	Cat#00-5521-00

Table S4. Reagents for Memory T Cell Analysis. Reagent, vendor, and catalog number are indicated.

Figure S1

ANTIGEN-SPECIFIC MEMORY B CELLS - PANEL 1



ANTIGEN-SPECIFIC MEMORY B CELLS - PANEL 2

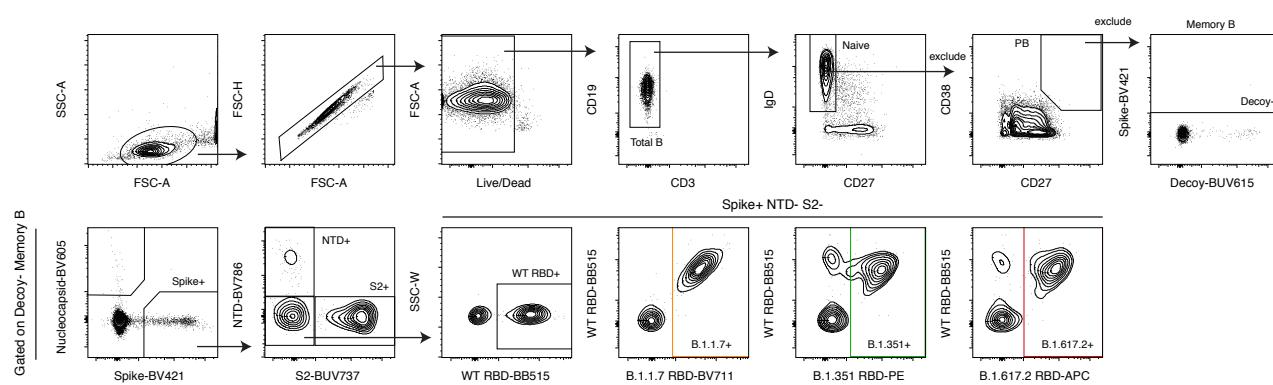


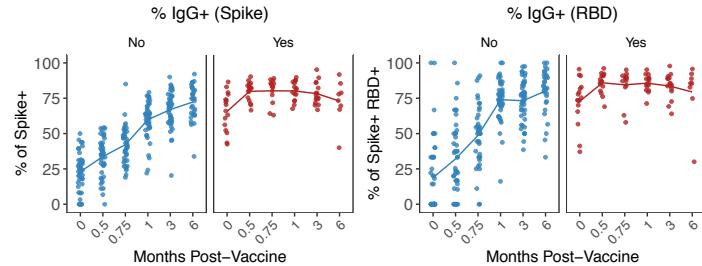
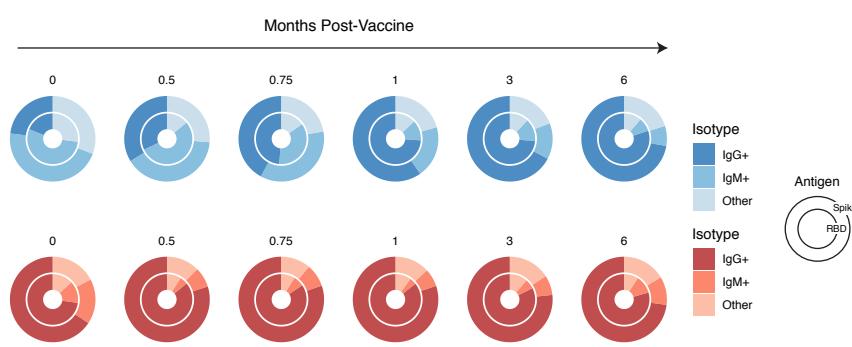
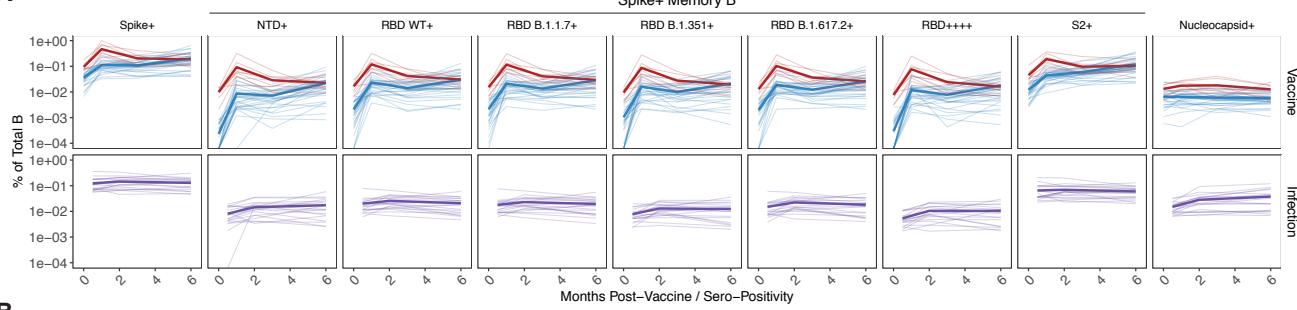
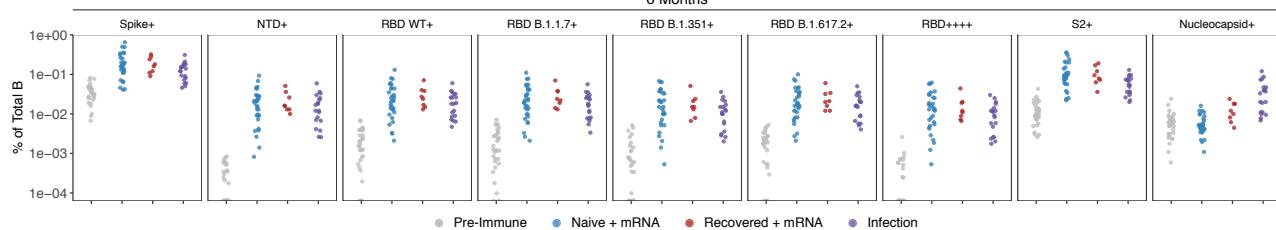
Figure S2**A****B****C**

Figure S3

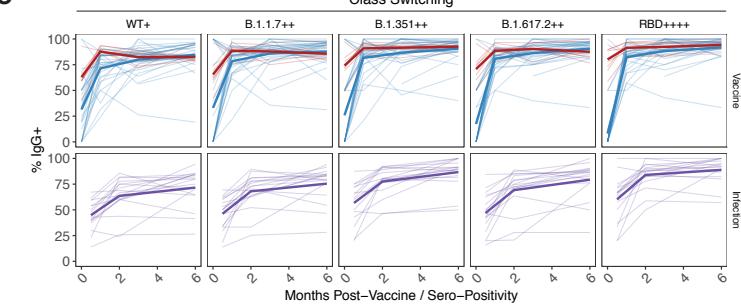
A



B



C



D

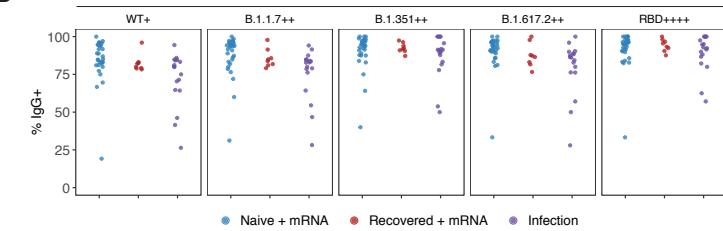
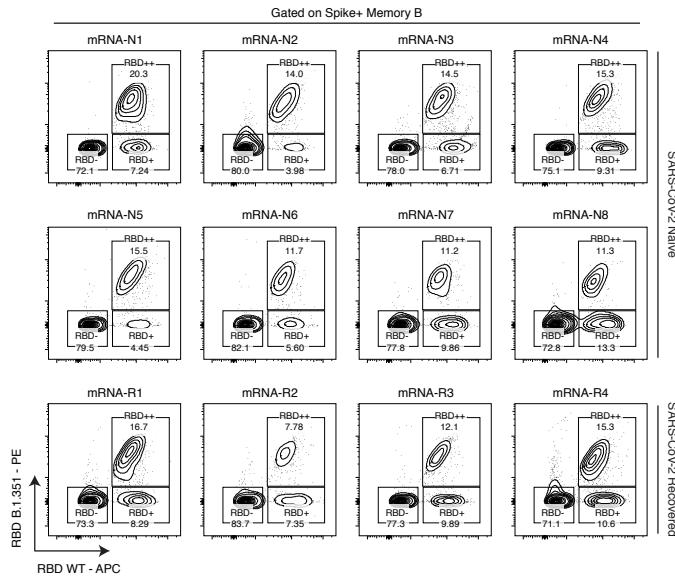
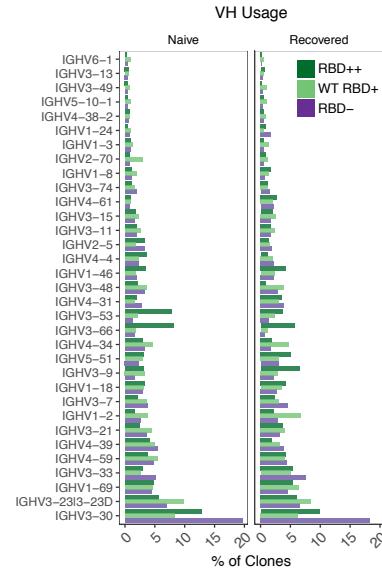


Figure S4

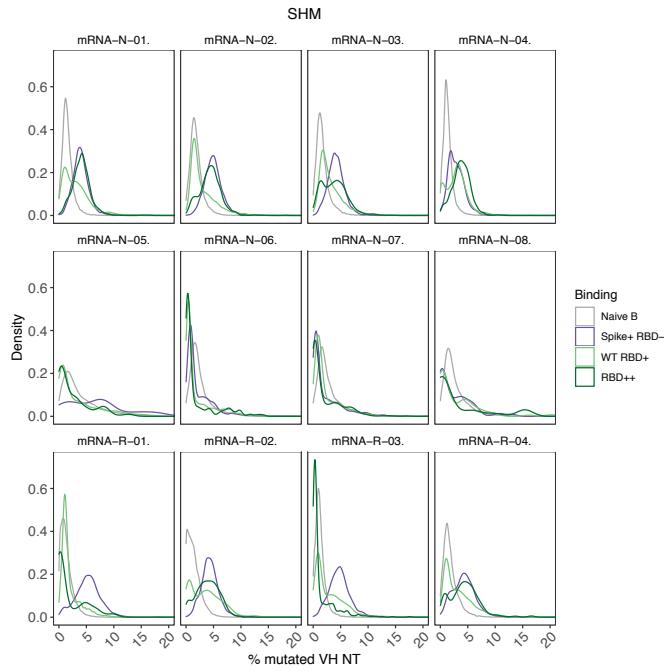
A



B



C



D

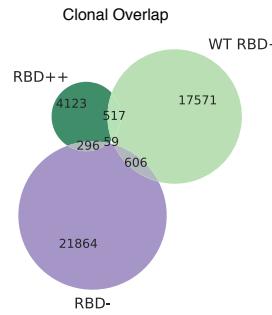


Figure S5

ANTIGEN-SPECIFIC T CELLS

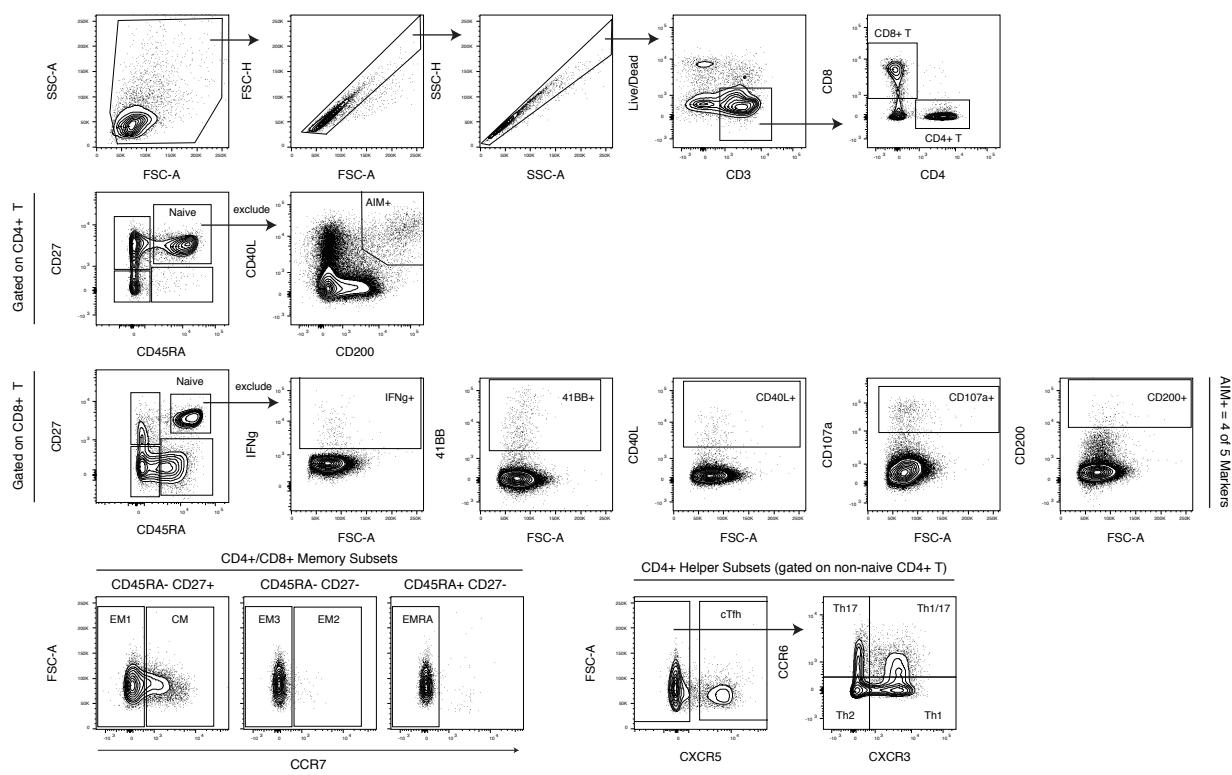
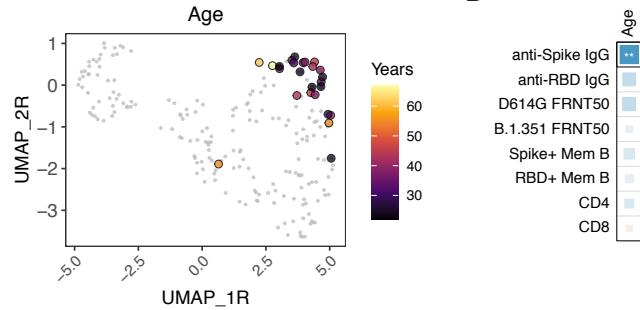


Figure S6

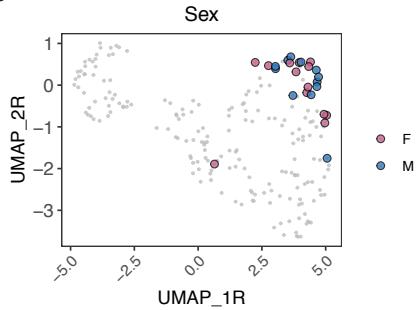
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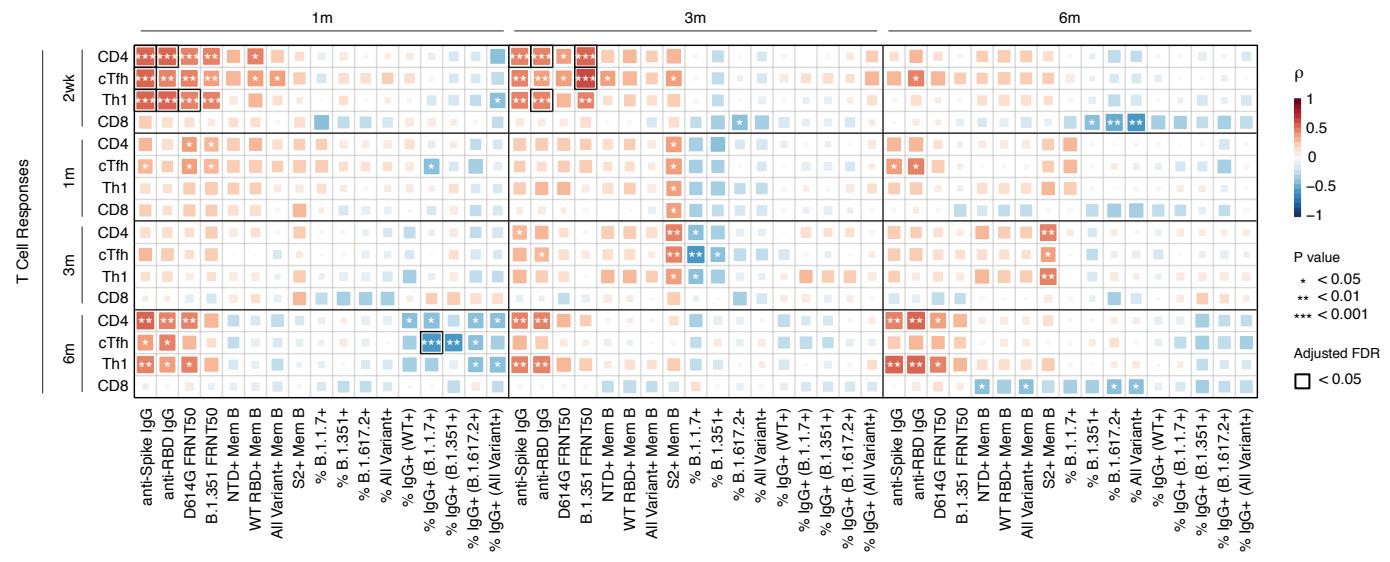
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C



D



E

